## Remarks

Claims 26-50 are pending.

Applicants gratefully acknowledge the withdrawal of the previous rejection of claims 26-34, 36-40 under 35 U.S.C. § 103(a) as being unpatentable over Brooks et al., Roberts et al., and Kimel et al.

Applicants also gratefully acknowledge the withdrawal of the previous rejection of claims 26-40 under 35 U.S.C. § 103(a) as being unpatentable over Brooks et al. and Rizzo et al.

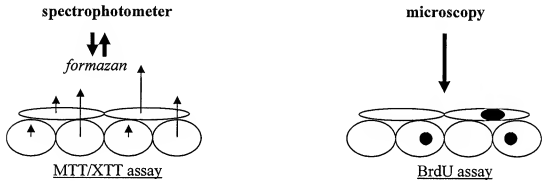
Applicants also gratefully acknowledge the withdrawal of the previous rejection of claims 26-50 under 35 U.S.C. § 101 as allegedly claiming the same invention as that of claims 1-2, 7-22 and 27-35 of co-pending Application No. 11/014,472.

## Rejection Under 35 U.S.C. § 103

**A.** The rejection of claims 41-50 is maintained under 35 U.S.C. § 103(a) as being unpatentable over Brooks et al. (Methods in Molecular Biology. 129:257-269), Kurz et al. (Dev. Dyn. 1995. 203:174-186), Frasca et al. (Oncogene. 2001. 20:3845-56), and Kinnman et al. (Lab Invest. 2001. 81(12):1709-16). The Office Action recognizes that while Brooks et al. described the potential of measuring angiogenic and anti-angiogenic activity using CAM assays, they did not teach adding an agent to measure metabolic activity. However, according to the Office Action, the use of proliferation-based assays was routinely used in the art to quantitate angiogenic or anti-angiogenic activity. The Office Action supports this position by pointing to the use of BrdU labeling in CAM assays as taught by Kurz et al. The Office Action then cites Frasca et al. and Kinnman et al. to support the position that metabolic markers such as XTT are interchangeable with BrdU as viable means of detecting cell proliferation. However, this assertion is incorrect.

Applicants noted in the response to the August 24, 2007 Office Action that the person of ordinary skill in the art at the time the application was filed would not have presumed that XTT could substitute for BrdU for detecting proliferation in the CAM. While it is true that BrdU detection is a proliferation assay, and that Kurz et al. teach the use of this method in CAM

assays, there is a significant difference between BrdU-based assays and the metabolic assays as claimed. Whereas BrdU can be used with a cell-specific antibody to discriminate the cell type, XTT is indiscriminate. As noted by the Examiner, XTT/MTT assays are based on the ability of metabolically active cells to reduce tetrazolium salt (MTT/ XTT) to a colored compound of formazan that is water soluble wherein the dye intensity can be read at a given wavelength with a spectrophotometer. Unlike BrdU, the colored formazan is not detected by an antibody or discernable by microscopy. As such, this method is an analysis of every cell in the assay. The following illustration demonstrates these differences:




Note that the measurement of formazan in MTT/XTT assays by a spectrophotometer does not discriminate which cells are metabolically active. In contrast, BrdU uptake by proliferating cells (like $^{3}$H-thymidine uptake) can be detected using microscopy such that the skilled artisan can visually determine whether the BrdU-labeled cell is the cell-type of interest (e.g., endothelial cell). MTT/XTT assays were therefore generally restricted in the art to homogeneous populations of cells. For example, Frasca et al. used XTT to measure proliferation of human thyroid cancer cells in matrigel, and Kinnman et al. investigated proliferation of hepatic stellate cells. Importantly, both of these reports were for homogenous cell cultures and not heterogeneous tissue.

In contrast, there are several additional cell types present in the CAM other than the endothelial cells making up the blood vessels. For CAM assays, therefore, the skilled artisan would have believed it necessary to account for the non-endothelial cells when assaying for

angiogenic effects. This is the reason why Kurz et al. analyzed BrdU labeled endothelial cells by computer assisted microscopy, which allowed a visual determination of whether a BrdU-labeled cell was in fact an endothelial cell.

The Office Action, however, alleges that "[t]here is no evidence on record that any other cell type that does not contribute in the process of angiogenesis is present in the capillary region of the CAM in significant number." Applicants therefore submit a manuscript from Dr. Judah Folkman's lab demonstrating evidence to this effect (Ausprunk DH, Knighton DR, Folkman J. Differentiation of vascular endothelium in the chick chorioallantois: a structural and autoradiographic study. Dev Biol. 1974 Jun;38(2):237-48, **attached hereto as Exhibit A**). Applicants specifically direct the Examiner's attention to Figure 11 on page 246, wherein autoradiographs of the CAM after exposure to tritiated thymidine were analyzed to evaluate endothelial cell proliferation at different stages of embryonic development. Notably, in addition to the endothelial cells (see arrows), there were also chorionic ectodermal cells, connective tissue cells (e.g., fibroblasts), and blood cells. More importantly, one can clearly see non-endothelial cells labeled based on exposure to tritiated thymidine. This highlights the reason Dr. Folkman chose a method of detecting proliferation that could be analyzed by microscopy. There was clearly a presumed need to visually determine if the proliferating cells were in fact endothelial cells.

Applicants therefore demonstrated surprising results in showing that metabolic substrates, such as XTT, which do not discriminate between cell types, could be used in the CAM assay to monitor the affect of angiogenic and anti-angiogenic agents on proliferation. Specifically, Applicants demonstrated that angiogenic stimulus can be used in combination with a metabolic assay to evaluate candidate anti-angiogenic agents in the CAM. There was no evidence in the cited art prior to the present results that such a non-discriminatory method could be used to detect significant effects of angiogenic and anti-angiogenic agents in a CAM. As such, the claimed method is not made obvious by the combination of Brooks et al., Kurz et al., Frasca et al. and Kinnman et al. Applicants therefore respectfully request the withdrawal of this rejection and allowance of claims 41-50.

**B.** Claims 26-40 are newly rejected under 35 U.S.C. § 103(a) as being unpatentable over Brooks et al. (Methods in Molecular Biology. 129:257-269), Iruela-Arispe et al. (Circulation. 1999; 100:1423-31), and Rizzo et al. (Microvascular Res., 1995, 49:49-63). The Office Action recognizes that while Brooks et al. described the potential of measuring angiogenic and anti-angiogenic activity using CAM assays, they did not teach directly injecting into CAM vessels a fluorescent-labeled particle to quantify fluorescent vascular density. The Office Action then attempts to cure this deficiency by citing Iruela-Arispe et al., who allegedly teach a method of measuring the effect of a test agent on angiogenesis in a modified CAM assay involving injecting FITC-dextran into the circulation of the CAM and measuring capillary density. However, as noted by the Office, Arispe et al. does not disclose capturing three dimensional pictures and digitally quantifying the plurality of pixels to obtain a fluorescent vascular density (FVD) as claimed. The Office then attempts to correct this deficiency by citing Rizzo et al. According to the Office Action, Rizzo et al. disclose a method to quantitate the relative microvascular permeability associated with tumorigenesis and normal angiogenesis by microinjecting a graded series of FITC-dextrans into a vessel of the CAM and then measuring the fluorescence by a confocal microscopy, which collects images at multiple tissue planes. The Office alleges that these results provide evidence that a method of capturing from the test region a 3-dimensional image comprising a plurality of pixels and digitally quantifying the plurality of pixels to obtain the fluorescence density was known in prior art to investigate the vasculature. Thus, according to the Office, it would have only required routine experimentation to modify the method disclosed by Iruela-Arispe et al., Brooks et al., and Rizzo et al. to arrive at the claimed method. Applicants respectfully disagree.

The skilled artisan would have had no reason to believe that quantitative analysis of a three-dimensional digital image of the vasculature within the CAM would provide superior results over a two-dimensional method. For example, Iruela-Arispe et al. injected FITC-dextran into the circulation of the CAM to evaluate vertical vessel growth into a collagen gel placed on top of the CAM by collecting a two-dimensional image of the collagen gel and measuring overall fluorescence. The skilled artisan would have had no motivation to collect a three dimensional

image of these blood vessels, since there was no reason to believe that a three-dimensional image would provide any additional information, and this motivation was not provided by Rizzo et al.

As noted by the Office Action, Rizzo et al. actually teach a method to quantitate vascular permeability associated with tumorigenesis and normal angiogenesis by microinjecting a graded series of FITC-dextrans (i.e., small enough to extravasate from leaky blood vessels) into a vessel of a CAM. The FITC-dextrans would therefore remain inside non-permeable vessles and, to a lesser degree, inside those that are leaky. As such, the artisan has to restrict analysis to the interstitial areas and exclude fluorescence from the blood vessels in order to evaluate extravasation using this method. Rizzo et al. therefore evaluated "interstitial optical intensity due to FITC-dextran extravasation ... by computer-assisted image analysis" (see abstract).

In contrast, the skilled artisan would know to use in the claimed method, as well as the method disclosed by Iruela-Arispe et al., FITC-dextrans that large enough to primarily remain inside the blood vessel during the assay. As such, there would have been no motivation to use the method disclosed in Rizzo et al. to restrict fluorescent detection to something less than the entire test area. This is not to say that the skilled artisan would not be so motivated in view of the instant disclosure, but prior to the filing date of the instant application, there was no indication that a three-dimensional analysis would be needed to restrict detection areas or to provide any additional information in order to establish the vascular density.

Consequently, the Examiner is using impermissible hindsight to suggest that the person having ordinary skill would have been motivated to improve the CAM assay disclosed in Iruela-Arispe et al. with three-dimensional analysis. Moreover, this approach provides unexpectedly superior results that were not anticipated by Brooks et al., Iruela-Arispe et al., and/or Rizzo et al.

In the response filed March 19, 2008, Applicants submitted a Declaration under 37 C.F.R. § 1.132 by Dr. Frank Cuttita, who is Director of the Angiogenesis Core Facility at the National Cancer Institute. Dr. Cuttitta states in his Declaration that the claimed quantitative CAM technology provides substantially superior results to that of Brooks et al. or any other previously available CAM assay. He indicates that the superior results of the claimed method were surprising to scientists in this field when they were first disclosed. While some

improvement of accuracy was expected with the use of more quantitative measurements of angiogenesis, Dr. Cuttitta states that the significance of the improvement shown by the claimed method was unexpected. In fact it was the surprising degree of improvement by the present method that first alerted those in the art to the scope of the problem with the prior art CAM assay. Dr. Cuttitta further states in his Declaration that the claimed assay has been commercially successful based on the recognition of the superior results obtained using the objective and direct quantitation of vessel density. He indicates that the results obtained with the claimed method have been lauded by others in the field in the face of competing methods and that this commercial success is directly related to the superior results of this assay over the methods previously used.

In view of these surprising results and the lack of motivation in the art prior to the instant disclosure to use quantitative three-dimensional analysis with the CAM assay, Applicants respectfully request the withdrawal of this rejection and allowance of claims 26-40.

**C.** Claims 41-50 are newly rejected under 35 U.S.C. § 103(a) as being unpatentable over Brooks et al. (Methods in Molecular Biology. 129:257-269), Kurz et al. (Dev. Dyn. 1995. 203:174-186), Yasukaawa et al. (Invest. Opthalmology Vis. Sci., 1999, 40:2690-96) and Woltering et al. (US Patent No. 6, 893,812). The Office Action again recognizes that while Brooks et al. describe the potential of measuring angiogenic and anti-angiogenic activity using CAM assays, and Kurz et al. describe the use of BrdU labeling in CAM assays, neither of these references teach the use of an agent to measure metabolic activity in the CAM assays. In order to overcome these deficiencies, the Office cites Yasukawa et al. and Woltering et al. as additional evidence that metabolic markers such as XTT and MTT are interchangeable with BrdU as viable means of detecting cell proliferation. Applicants respectfully disagree with this assertion and traverse this rejection for the reasons stated above in Section A. Applicants demonstrated surprising results in showing that metabolic substrates, such as XTT, which do not discriminate between cell types, could be used in the CAM assay to monitor the affect of angiogenic and anti-angiogenic agents on proliferation.

Moreover, Yasukawa et al. is being cited for use of tetrazolium-based colorimetric system to measure human umbilical vein endothelial cell (HUVEC) growth. Applicants note that HUVECs are a homogeneous cell line and not a heterogeneous tissue as discussed above. Likewise, while Woltering et al. disclosed the use of MTT assays in their angiogenesis assay involving placement of a tumor discs into a matrix, they do not teach 1) that it has any utility in measuring proliferation or 2) that it could be used in a heterogeneous tissue. First, they recite MTT assays for measuring "cellular viability" rather than proliferation. Instead, they teach that proliferation can be measured by $^{3}$H-thmidine uptake, etc… Moreover, they indicate that tissue-versus-vessel uptake would require "specific receptor-mediated tags" without giving any examples or other guidance. Thus, Woltering et al. clearly recognized that the assay would be non-discriminatory and not give data specific for angiogenesis, since the tumors would likely be proliferating also. Applicants therefore respectfully request the withdrawal of this rejection and allowance of claims 41-50.

**Double Patenting Rejection**

Claims 26-50 were provisionally rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 1, 4-34 of co-pending Application No. 11/014,472. Applicants acknowledge this rejection and will formally respond to the provisional double patenting rejection in the appropriate application once claims are found to be allowable necessitating the removal of the provisional status of the rejection.

**Conclusion**

Pursuant to the above amendments and remarks, reconsideration and allowance of the pending application is believed to be warranted. The Examiner is invited and encouraged to directly contact the undersigned if such contact may enhance the efficient prosecution of this application to issue.

A Credit Card Payment submitted via EFS WEB authorizing payment in the amount of $490.00, representing the fee for a large entity under 37 C.F.R. § 1.17(a)(2) for a Two Month Extension of Time, a Request for Extension of Time, and Exhibit A (Ausprunk DH, et al. Dev Biol. 1974 Jun; 38(2):237-48) are hereby enclosed. This amount is believed to be correct; however, the Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 14-0629.

Respectfully submitted,

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| Signature | Brian Giles | Date | 10-7-2008 |